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Two transcriptional start sites found in the promoter region of *Escherichia coli* glutamine permease operon, *glnHPQ*

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Presence of the two different types of promoter in *Escherichia coli glnHPQ* operon has been suggested by the deletion study using Φ(*glnH*'-'*lacZ*') gene (1). Here we determined exactly the 5'-end of mRNA by primer extension. Total RNA was prepared from the TNK23 host (F^- *thi metC* Δ(*glnQPH-chlE*) Δ(*lac*) X74 *aroL*) harboring pTN118 mini-F plasmid. This plasmid contains 1.4 kb *Xho*II fragment of pTN256 which carries 671 bp 5'-flanking region of *glnH* and the first 22 bp of *glnH* structural gene fused to the 20th bp of *lacZ*' gene (1). The ^{35}S-labeled primer hybridized to the RNA was extended by reverse transcriptase, and the products were analyzed in the buffer gradient gel with the dideoxy sequencing ladders as the size markers. Two clear bands could be seen (data not shown); one (T1) started at the -194th G and the other (T2) at the -43rd G (Fig. 1). The canonical *E. coli* promoter sequence (2) could be assigned for both the upstream and the downstream promoters, *glnHp1* and *glnHp2*. The *glnHp2* region also has homology to Ntr and Nif promoters (3,4), which are recognized by the novel σ^{60} factor (*rpoN* product) of RNA polymerase (5,6) in place of the major σ^{70} factor.

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         .         . glnHp1  .      -201    T1   .         .         .         .      -151         .
tacataaagaTTGTTTttcatcaggttttacgCTAAATaatcactGtgttgagtgcacaattttagcgcaccagattggtgccccagaatggtgcatct
                                             *→

         .         .         .      -101         .         .         . glnHp2  .      -51      T2 .
tcagggtattgccctataaatcgtgcatcacgttttgccgcatctcgaaaaatcaaggagTTGCAAaaCTGGCACGatttTTTCATATatgtgaatGtc
                                                                                                *→

         .         .         .      -1 glnH→    .          .   lacZ→    .        .
acgcaggggatcgtcccgtggatagaaaaaaggaaatgct atg aag tct gta tta aaa gtt tca ctg gcc gtc gtt tta caa cgt
                               S.D.                                   ←t gac cgg cag caa aat gtt gc
                                                                            (Primer)
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Fig. 1. Nucleotide sequence of the promoter region of Φ(*glnH*'-'*lacZ*') gene in pTN118 and pTN256. Asterisks show two transcriptional start sites, T1 and T2. The *glnHp1* and *glnHp2* promoter sequences are indicated by capitals.

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